Project Title	Liverwort control using novel techniques
Project number:	HNS 175
Project leader:	Jill England, ADAS Boxworth
Report:	Year 1 report, April 2010
Key staff:	Dr Jill England - Project leader and principal investigator John Atwood (ADAS) - Specialist advisor Dr Peter Fletcher (NIAB) - glucosinolate analysis (subcontractor) Chris Dyer – ADAS Statistician
Location of project:	Oakover Nurseries, Ashford, Kent Palmstead Nurseries, Wye. Kent
Project coordinator:	John Richards, John Richards Nurseries Dr Neal Wright, Micro Propagation Services
Date project commenced:	1 April 2009
Date completion due:	March 2012
Key words:	Liverwort, glucosinolate hydrolysis product, growing media amendment, suppression, growing media, *Marchantia polymorpha*

The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Jill England
Horticultural Consultant
ADAS

Signature ..... Date .....

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Grower Summary

Headline

- Addition of seed meals *Sinapis alba*, *Brassica napus* '00' and *Camelina sativa* can significantly reduce liverwort establishment.
- Growing media amendment with Sylvafibre® and sterilised loam can significantly reduce liverwort establishment.

Background and expected deliverables

Liverwort growing on the surface of growing media is a major problem to the horticulture industry, affecting both protected and outdoor grown hardy nursery stock; the cost of hand removal of moss, liverwort and weeds at dispatch has been estimated at 4% of total annual production costs, equating to £1,763 per hectare based on 2008-9 figures. Zero tolerance of liverwort in certification schemes and a lack of approved chemical products also make its control a technical priority for growers.

The aim of this project is to build on work completed in HDC projects HNS 126 and HNS 93c by investigating further the herbicidal effect of glucosinolate (GSL) hydrolysis products found in oilseeds on liverwort, and the suppression of liverwort growth by unknown biological or physical factors within certain growing media components.

GSLs and their hydrolysis products (isothiocyanates, ITCs) are responsible for the distinctive pungent smell and hot taste of cabbages, mustards and other brassicas and are known to have toxic effects against plants, root knot nematodes and fungal species; brassicas are also successfully used in the bio-fumigation of soils against weeds and diseases.

ITCs are the most bioactive products of GSL hydrolysis and have been shown to exhibit a herbicidal effect on liverwort; ITCs adversely affect liverwort gemmae (vegetative propagules produced by gemma cups on the liverwort surface) comparable to commercially used herbicides (lenacil and metazachlor) when tested under laboratory conditions (HDC project HNS 126). *Limnanthes alba* seed meal provided short-term liverwort control when incorporated into growing media (HDC project HNC 93c), and *Sinapis alba* 'IdaGold' applied as a mulch has been found to control established liverwort.

Observations made by ADAS consultants during project HNS 93c suggested a suppressive effect on liverwort growth where the growing media was amended with loam or Sylvafibre®, possibly indicating natural microbial suppression in addition to any physical effect. Work carried out under the auspices of the Peatering Out project similarly suggested a suppressive effect of green compost on liverwort growth. Suppression of liverworts through microbial activity from growing media amendments has not been investigated but represents an opportunity to improve control if a better understanding of the effects can be obtained.

The expected deliverables from this work include the development of an effective novel control for liverwort infestation based on:

- Growing media amendment with seed meal or a combination of seed meals to reduce liverwort establishment.
- Growing media amendment with materials to provide natural microbial suppression of liverwort in addition to any physical effect.

Summary of the project and main conclusions

Two trials were carried out during 2009/10, investigating seed meal and growing media suppressive effects on liverwort establishment and growth. Both trials were carried out under protection.

Seed meal suppressive effect

Five oil seeds (*Brassica carinata, Sinapis alba, Camelina sativa* and two different *Brassica napus* (oilseed rape samples) were selected for inclusion in this trial, aiming to include products grown as commercial crops in the UK, where the seed meal was a waste product, and which would provide a range of glucosinolates to test.

The seed meals were processed to a fine meal and analysed for glucosinolate content. Each was applied both as a mulch and incorporated into the growing media at a rate of 3% to investigate the effect of application method. A pot of established liverwort was placed within each plot to provide liverwort inoculum.

Liverwort establishment was least in the *Sinapis alba* (incorporated), *Camelina sativa* (mulch) and *Brassica napus* '00' treatments after 19 weeks (Figure 1). Of these, the results for *Sinapis alba* were most consistent, with least variability in the amount of liverwort established. During the winter period the trial became excessively dry as automatic irrigation

was not used. In addition to this, a fungal infestation which had been noted previously, spread throughout the liverwort in the trial. The combination of these two factors adversely affected liverwort development at the end of the trial. (It is possible that the fungal infestation was opportunistic as the liverwort was under stress or it could have been a primary pathogen of liverwort, which would warrant further investigation). Data collected after 30 weeks reflected these events and any effects of the seed meal were not clear.

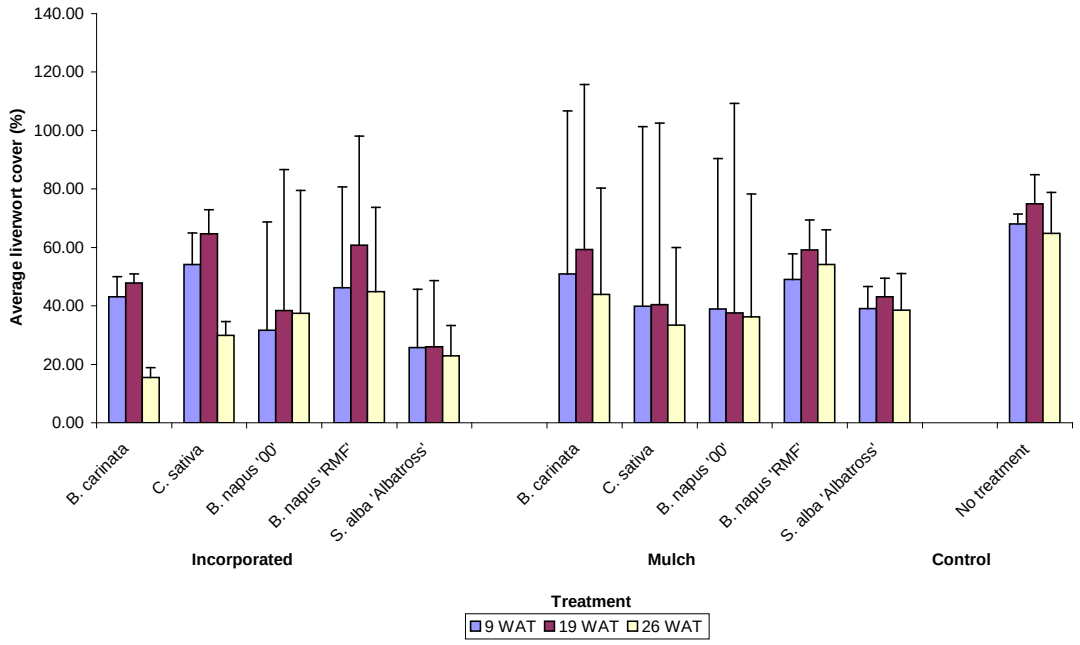


Figure 1: Seed meal suppressive effect (WAT = weeks after treatment)

Future research could investigate optimum application rates and combining seed meals to provide higher levels of liverwort control, along with examining any potential phytotoxic effects against crop plants.

Growing media suppressive effect

Five products were included in the trial (Melcourt Sylvafibre®, Melcourt Growbark®, Perlite, Vital Earth Green Compost and sterilised loam), with Sinclair Professional Peat used as a base. Treatments were incorporated into the peat at a standard rate of 50%, except for the sterilised loam (20%); 50% loam would not be used commercially by growers due to the increased weight of the media. Treatments were watered by hand in addition to overhead irrigation to maintain high water levels and exclude any physical effects due to improved drainage. This also served to increase liverwort pressure. Trays were placed on a mypex-covered bed topped with gravel. Again, a pot of established liverwort was placed within each plot to provide liverwort inoculum.

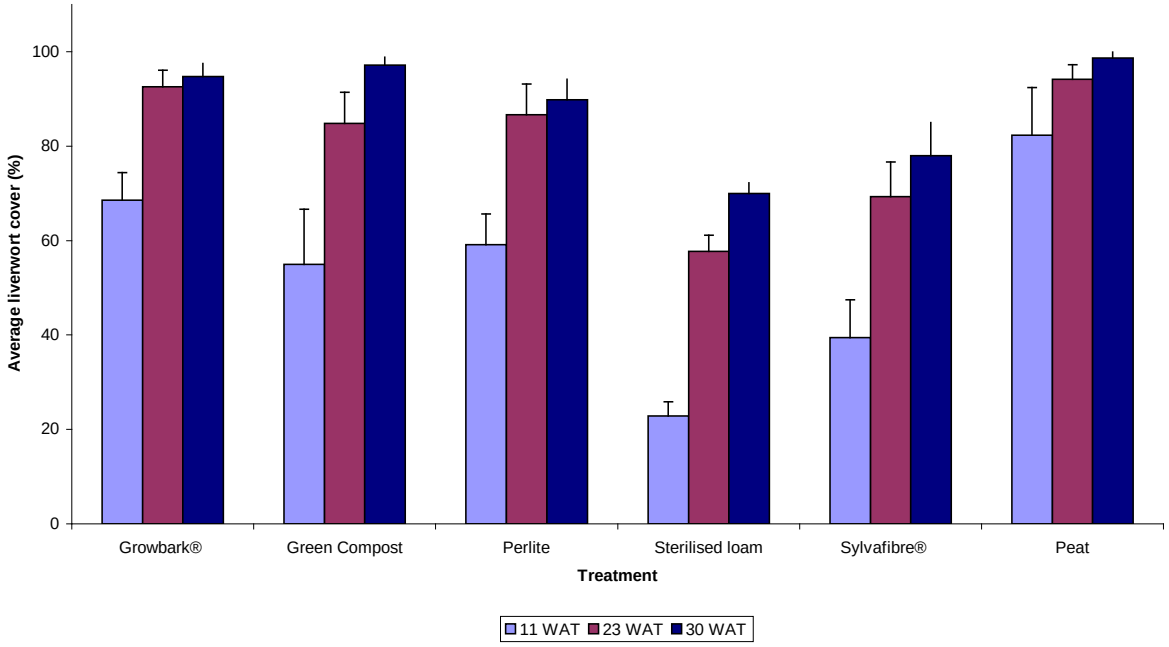


Figure 2: Growing media suppressive effect (WAT = weeks after treatment)

Peat treatments (Figure 2) had a high level of liverwort infestation from early in the trial, with 99% coverage after 30 weeks. Liverwort was slow to establish in the green compost treatment, but after 30 weeks liverwort cover was comparable to that seen in the peat treatments. The Growbark® and perlite treatments also had a high level of liverwort throughout the trial. It is normally expected that the increased drainage provided by these products leads to reduced liverwort cover due to the drier growing media surface, but as high moisture levels were maintained by additional hand watering this effect was eliminated from the trial.

Both Sylvafibre® and sterilised loam had a significant effect, with less liverwort cover in these treatments. Liverwort was slow to establish in the Sylvafibre® treatments although 78% liverwort cover was recorded after 30 weeks. The beneficial effect of Sylvafibre® had previously been attributed to the improved drainage imparted on growing media, but these results suggest that other factors may also be implicated.

Throughout the trial the sterilised loam showed least liverwort establishment compared to all other treatments. Whilst this may show promise in reducing liverwort infestation, the weight and cost of loam may restrict the proportion that could be included in commercial growing media. Future work could investigate the combined effect of these treatments and irrigation levels.

Financial benefits

- The growing media amendment treatments could reduce the need for hand cleaning pots at dispatch. The cost of moss, liverwort and weed removal by hand at dispatch is estimated to be 4% of the total annual production costs, equating to £1,763 per hectare based on 2008-9 production figures.
- The treatments could reduce the need for specific herbicidal liverwort treatments during production. For example, Venzar Flowable costs £105 per hectare or Clayton Lenacil costs £140 per hectare (these figures are in addition to the cost of liverwort removal at dispatch).
- High levels of control would mean that plants free from liverwort infestation could be offered to customers.

Action points for growers

- Growers could consider including a proportion of Sylvafibre® or sterilised loam into potting mixes to aid liverwort reduction, particularly in the case of shorter term crops.
- Further investigations of the effects of seed meal on both liverwort and crop plants are required before any specific recommendations can be made to growers.

Science Section

Introduction

Liverwort growing on the surface of growing media is a major problem to the horticulture industry, affecting both protected and outdoor grown hardy nursery stock; their removal has been estimated at 4% of total annual production costs. Zero tolerance of liverwort in certification schemes and a lack of approved chemical products make its control a technical priority for growers.

The aim of this project is to build on work completed in HDC projects HNS 126 and HNS 93c by investigating further the herbicidal effect of glucosinolate hydrolysis products found in oil seeds on liverwort, and the suppression of liverwort growth by unknown biological or physical factors within certain growing media components.

Objectives

1. To investigate the use of brassica seed meal products (containing glucosinolates) applied as a mulch and incorporated into growing media, to control liverwort.

2. To investigate the suppressive effect of growing media amendments on liverwort establishment and growth.

Objective 1: Seed meal suppressive effect

Glucosinolate (GSLs) and their hydrolysis products (isothiocyanates, ITCs) are responsible for the distinctive pungent smell and hot taste of cabbages, mustards and other brassicas and are known to have phytotoxic effects (e.g. against *Amaranthus palmeri* (Bialy et al., 1990) as well as toxicity against root knot nematodes and fungal species. GSLs could potentially be used to control weeds; each brassica variety has a distinctive profile of one or more glucosinolates, each of which could have a different effect on liverwort and other weeds.

GSLs are non-toxic thioglucosides with a common core comprised of a β-D-thioglucose group with a sulphonated oxime and a variable side chain ('R' group) that largely determines the biological activities of the degradation products (Figure 3) (Brown and Morra, 1999). GSL hydrolysis (Figure 4) is catalysed by a myrosinase enzyme released following mechanical damage in the presence of water; GSLs and myrosinase are stored separately within the plant and come into contact only following mechanical damage.

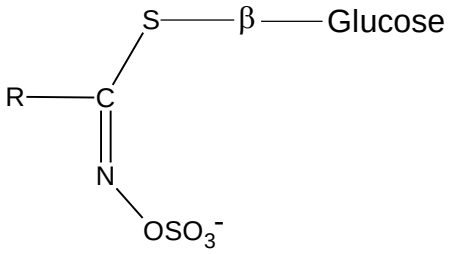


Figure 3. Structure of glucosinolate. R represents the variable side chain. Adapted from Mithen (2001).

The products of this reaction are primarily isothiocyanates (ITCs), thiocyanates, nitriles, or epithionitriles, depending on the 'R' group present and the environment. ITCs are the most bioactive of the hydrolysis products and more phytotoxic than the corresponding nitriles (Vaughn et al., 2006).

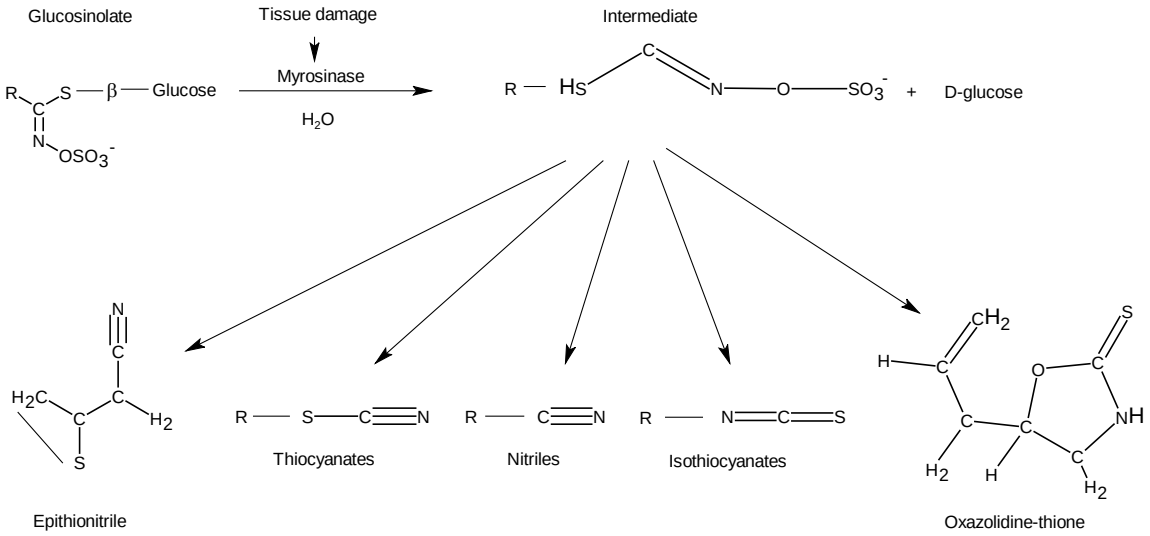


Figure 4. General structure of glucosinolates and their hydrolysis products. Adapted from (Vaughn and Berhow, 2005).

GSL hydrolysis products (2-phenylethylITC, 2-propenylITC, benzylITC and 3-methoxybenzylITC) have been shown to exhibit a herbicidal effect on liverwort gemmae (vegetative propagules produced by gemma cups on the liverwort surface), comparable to two herbicides (lenacil and metazachlor) when tested under laboratory conditions (Jeger, 2008). *Limnanthes alba* (Limnanthaceae, glucosinolate glucolimnanthin, 3-methoxybenzylITC) seed meal has been shown to provide short-term liverwort control when incorporated into growing media (Atwood, 2005). *Synapis alba* 'IdaGold', applied to the surface of growing media, has been found to control established liverwort from 83 to 97%, six weeks post treatment (Boydston et al., 2008). During this study five seed meals (*Brassica carinata, Sinapis alba, Camelina sativa* and two different oilseed rape samples) were selected and their effect on liverwort establishment and growth was measured.

Objective 2: Growing media suppressive effect

Observations made by ADAS consultants during project HNS 93c suggested a suppressive effect on liverwort growth where the growing media was amended with loam or Sylvafibre®, possibly indicating natural microbial suppression in addition to any physical effect (Atwood, 2005). Work carried out under the auspices of the Peatering Out project similarly suggested a suppressive effect of green compost on liverwort growth (Adlam and Rainbow, 2002). Suppression of liverworts through microbial activity from growing media amendments has not been investigated but represents an opportunity to improve control if a better understanding of the effects can be obtained. During this study the effects of five products on liverwort establishment and growth were compared: Melcourt Sylvafibre®, Melcourt Growbark®, Perlite, Vital Earth Green Compost and sterilised loam.

Materials and methods

Two trials were carried out during 2009/10, sited at Palmstead Nurseries (seed meal suppressive effect) and Oakover Nurseries (growing media suppressive effect), under polytunnel and glass respectively.

Objective 1: Seed meal suppressive effect

Five oil seeds (*Brassica carinata, Sinapis alba, Camelina sativa* and two different oilseed rape samples) were selected for inclusion in this trial. The aim was to select products grown as commercial crops in the UK, where the seed meal was a waste product, and which would provide a range of glucosinolates:

- *Camelina sativa* (false flax) is grown in the UK by Statfold Oils, Tamworth, Staffordshire, who extract the oil for the personal care, food and nutrition markets. *C. sativa* is reported to have an inhibitory effect on flax (*Linium usitatissimum*) seedling growth.
- *Brassica carinata* (Abyssinian mustard, Ethiopian mustard) is produced in the UK by Plant Solutions Ltd as Biofence, a product developed to contain high levels of glucosinolates, and reported to suppress weeds, mainly annuals, in addition to having a fumigant effect on pathogens.
- *Sinapis alba* 'Albatross' (syn. *Brassica alba, B. hirta*, white mustard) oil is extracted for the culinary market. It can be grown in the UK, but more often the crop is grown in warmer climates and the oil imported into the UK.
- Oilseed rape (*Brassica napus*) is widely grown in the UK to produce animal feed, culinary oil and biodiesel. It has been bred to reduce the glucosinolate content as this is toxic to animals. The samples used were obtained from two different sources, one (*Brassica*

napus '00') was a '00' variety (bred for low glucosinolate and erucic acid content) and the other, sourced from Romney Marsh Farms (*Brassica napus* RMF) contained seed from a number of oilseed rape varieties and provided a different glucosinolate profile (Appendix 1).

The seed meals were generally crushed, the oil extracted and then reformed into pellets, flakes or fine meal prior to supply. *Sinapis alba* 'Albatross' was only available as seeds, (Farm Direct, Cumbria) which were crushed and the oil extracted by Alan Brewer (Selby House Farm, cold extraction). Oil in seed meal tends to become rancid. All seed meal was processed to a fine meal before use in the trial. Seed meals were analysed for glucosinolate content by NIAB using test procedures based on British Standard BS 4289 Part 9: 1993 ISO 9167-1 1992 (Appendix 1) and their effect on liverwort establishment and growth was measured.

Experimental design

Treatments (Table 1) were arranged in a randomised block design with 4-fold replication (Appendix 2). Each plot consisted of a tray of 17 liners (9cm pots), with one additional pot containing liverwort to introduce inoculum; plants were not used in the trial.

Treatments

The seed meals were incorporated (I) or applied as a mulch (M), both at 3% application rate (Table 1). Trays were placed on a Mypex-™covered bed. Irrigation was provided by overhead sprinklers, and by hand watering during the winter when automatic irrigation was not used. The trial was set up on 21 September 2009.

The potting mix was comprised of:

- 100% Sinclair Professional Peat, medium/coarse
- Osmocote Exact Standard High K, 11:11:18 + 2 MgO + trace elements, 8-9 months @ 3 kg/m^3
- Lime to pH 5.5

Table 1. Seed meal suppressive effects treatments

Product	Supplier	Application rate
Brassica napus '00'	ADM Ingredients Ltd	3%
Brassica napus RMF	Romney Marsh Farms	3%
Camellina sativa	Statfold Seed Oil Ltd	3%
Sinapis alba	Farm Direct	3%
Brassica carinata	Plant Solutions Ltd	3%
No amendment	-	-

Assessments

Inspections were carried out as follows:

16 October 2009	3 WAT*	Inspection
24 November 2009	9 WAT	Assessment (% pot cover of liverwort)
6 January 2010	15 WAT	Inspection
1 February 2010	19 WAT	Assessment (% pot cover of liverwort)
26 February 2010	22 WAT	Inspection
26 March 2010	26 WAT	Assessment (% pot cover of liverwort)

*WAT = weeks after treatment

Objective 2: Growing media suppressive effect

Treatments

Five products were included in this trial (Table 2), with Sinclair Professional Peat used as a base. Treatments were incorporated into the peat at a standard rate of 50%, except for the sterilised loam (20%); 50% loam would not be used by commercial growers due to the increased weight of the media. Treatments were watered by hand in addition to overhead irrigation to maintain high water levels and exclude any effects due to improved drainage. This also served to increase liverwort pressure. Trays were placed on a Mypex™ covered bed topped with gravel.

Table 2. Growing media suppressive effect treatments

Product	Supplier	Application rate
Melcourt Sylvafibre®	Melcourt Industries Ltd	50%
Green Compost (<10 mm)	Vital Earth Ltd	50%
Standard Horticultural Perlite (2.0-5.0 mm)	Sinclair Horticulture Ltd	50%
Surrey Loam (sterilised, screened, 0.25")	Rigby Taylor	20%
Melcourt Growbark® (pine)	Melcourt Industries Ltd	50%
Sinclair Professional Peat (medium grade)	Sinclair Horticulture Ltd	100%

Nutrients were added as follows:

- Osmocote Exact Mini, 16-8-11 + 2 MgO + trace elements, 3-4 month @ 1 kg/m^3
- Dolomite lime @ 500 g/m^3. Lime was added to the Sylvafibre® treatment @ 1 kg/ m^3 as per the manufacturer's instructions. No lime was added to the green compost.

The trial was set up on 27 July 2009.

Experimental design

Treatments were arranged in a randomised block design with 4-fold replication (refer to Appendix 2 for layout). Each plot consisted of a tray of 17 liners (9cm pots), with one additional pot containing liverwort to introduce inoculum; plants were not used in the trial.

Assessments

Inspections were carried out as follows:

25 August 2009	4 WAT*	Inspection
16 October 2009	11 WAT	Assessment (% pot cover of liverwort)
18 November 2009	16 WAT	Inspection
6 January 2010	23 WAT	Assessment (% pot cover of liverwort)
1 February 2010	27 WAT	Inspection
26 February 2010	30 WAT	Assessment (% pot cover of liverwort)

*WAT = weeks after treatment

Results and Discussion

Objective 1: Seed meal suppressive effect

Over the whole trial least liverwort established in the *Synapis alba* (incorporated), *Camelina sativa* (mulch) and *Brassica napus* '00' (incorporated) treatments, and most liverwort established in the control (Figure 5 and Appendix 3). In all treatments except for the *Synapis alba* (incorporated) and *Brassica napus* '00' (mulch) the trend was for increased liverwort cover at each assessment. Of the two *Brassica napus* seed meals ('00' and RMF), *Brassica napus* '00' had a greater effect, with less liverwort establishing in these treatments.

During the winter period the nursery automatic irrigation was not used, instead plants were spot watered by hand across the nursery as is common practice during the winter. After 22 weeks many of the pots were extremely dry, with the liverwort suffering due to lack of water; irrigation was applied by hand for the remainder of the trial. The effect of the lack of water will have contributed to the decreased liverwort cover in all treatments at the final assessment after 26 weeks.

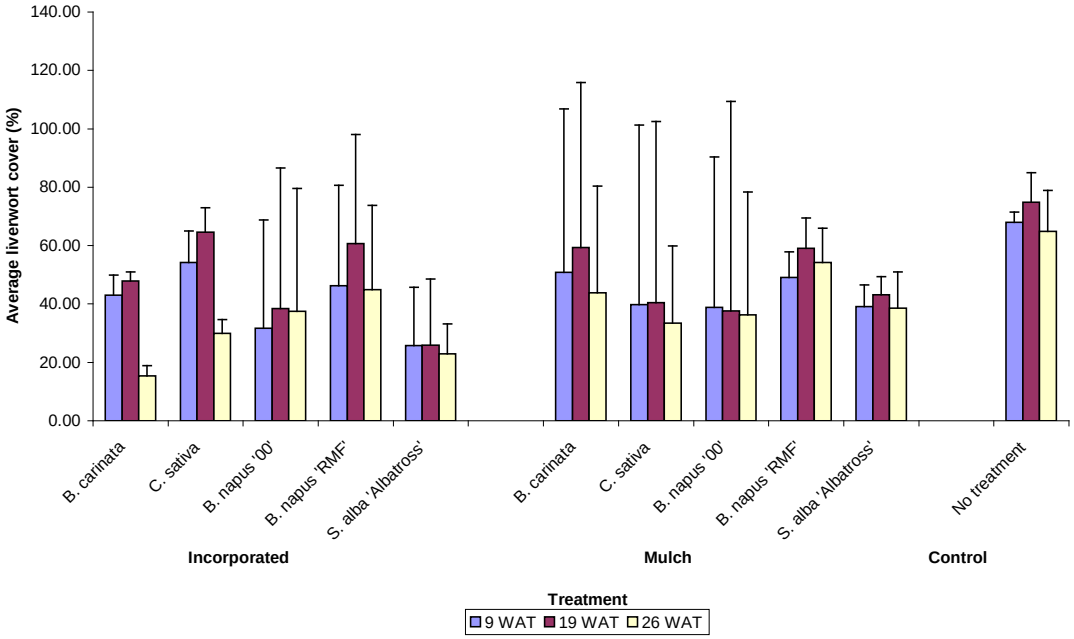


Figure 5. Seed meal suppressive effect (WAT = weeks after treatment)

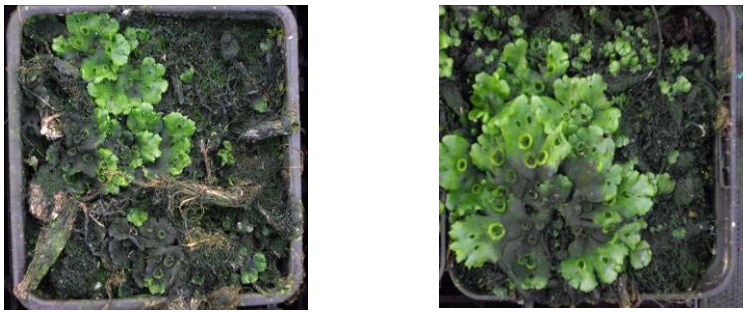
White fungal growth had been observed on the Mypex™ and in a number of pots in autumn 2009 (Figure 6) and by the final assessment this had spread throughout the trial (Figure 7). On close inspection it could be seen that the liverwort in a number of pots across the trial was dying, initially becoming black from the centre of the plant. New growth was evident in some pots. Samples of the fungal growth were passed to the Fera plant clinic for identification, but the results were inconclusive; ITS sequence data did not provide a reliable identification but suggested either *Verticillium leptobactrum* (a nematophagous fungus) or, more likely, a *Paecilomyces* species (a soil saprophyte).

***Camelina sativa* (mulch) (9 WAT)**

***Brassica carinata* (mulch) (19 WAT)**

Figure 6. Examples fungal growth in treatments

Figure 7. Seed meal trial (22 WAT)



***Synapis alba* (incorporated)** ***Brassica napus* RMF(mulch)**

Figure 8. Examples of dying liverwort (19 WAT)

Figure 9. Plot sited beneath the tunnel frame. (Note the wet growing media and line formed by dripping condensation).

Data collected after 26 weeks was subjected to statistical analysis, but was not found to be significant. However, statistical analysis of data collected after 19 weeks analysed using analysis of variance (Table 3) showed a highly significant difference in liverwort cover between treatments ($F_{4,27}$ = 5.06, P<0.05). Closer inspection of the data indicated that liverwort cover in the *Synapis alba* (incorporated and mulch), *Brassica napus* '00' (incorporated and mulch) and *Camelina sativa* (mulch) treatments was significantly less than in the control. However, liverwort coverage was highly variable in the majority of treatments, with the most consistent results found within the *Synapis alba* plots.

Table 3. Analysis of variance (ANOVA) comparing average liverwort cover, 01.02.10, 19 WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.	
Block	3	296.1	98.7	0.53	0.668	
Treatment	4	3779.5	949.9	5.06	0.004	**
Method	1	1.7	1.7	0.01	0.925	
Treatment.Method	4	2030.5	507.6	2.71	0.051	
Residual	27	5064.4	187.6			
Total	38	11192.2				

Although statistical analysis using data gathered after 19 weeks (Table 3) did not identify a significant difference due to application method ($F_{4,27}$ = 2.71, p=0.051) it did suggest a trend towards less liverwort establishment where treatments were applied as a mulch (Figure 10). When the final assessment, after 26 weeks, was discounted liverwort cover was reduced in the mulched compared to the incorporated treatments. After 19 weeks there was less liverwort cover in the mulch rather than incorporated *Brassica napus* RMF, *Brassica napus* '00' and *Brassica napus* '00' treatments; however less liverwort cover developed in the incorporated *Synapis alba* and *Brassica carinata* treatments.

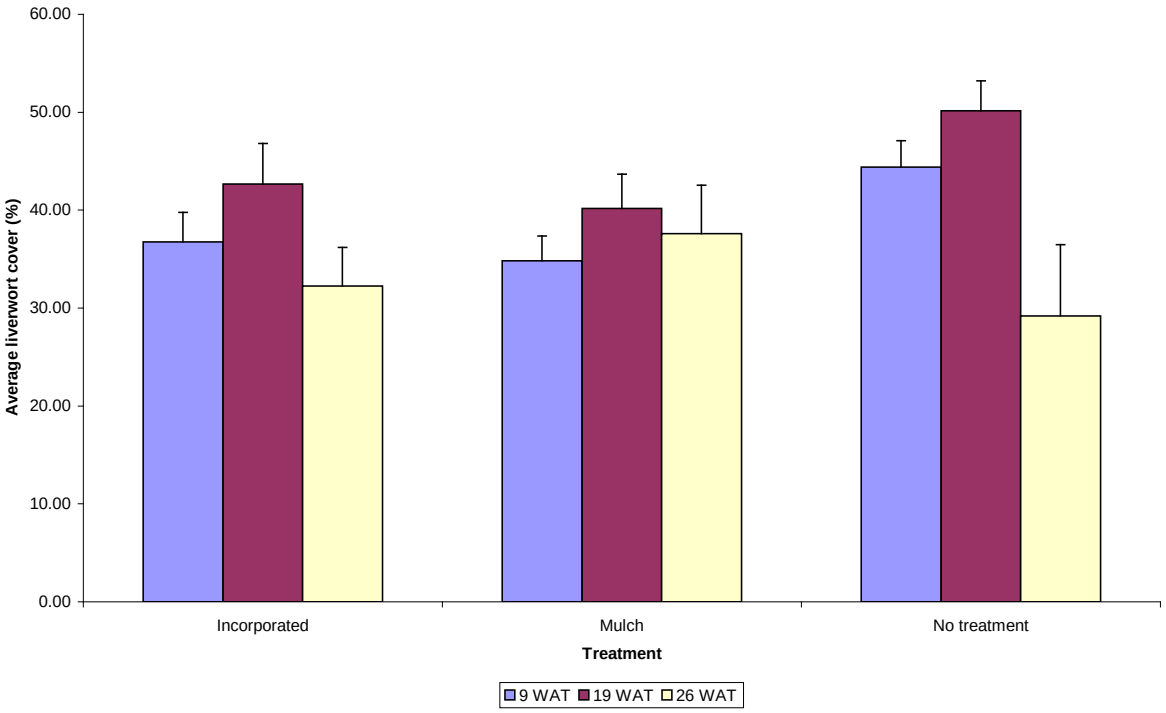


Figure 10. Comparison of application method (WAT = weeks after treatment)

A number of plots sited directly beneath the tunnel frame were wetter than the rest of the trial, where condensation from the frame had dripped onto the pots beneath (Figure 9), and these generally had greater liverwort growth. The data were re-evaluated omitting data from plots sited beneath the tunnel frame.

As previously, data collected after 26 weeks was not found to be significant. However, statistical analysis of data collected after 19 weeks analysed using analysis of variance (Table 4) showed a highly significant difference in liverwort cover between treatments ($F_{4,27}$ = 7.33, P<0.001). Closer inspection of the data indicated that liverwort cover in the *Synapis alba* (incorporated and mulch), *Brassica napus* '00' (incorporated and mulch), *Brassica napus* RMF (mulch), *Camelina sativa* (mulch) and *Brassica carinata* (incorporated) treatments was significantly less than in the control. However, liverwort coverage remained highly variable in the majority of treatments, with the most consistent results found within the *Synapis alba* plots (Figure 11).

The statistics indicated that there was an interaction between treatment and application method after 9 ($F_{4,20}$ = 3.07, p=0.040) and 19 weeks ($F_{4,20}$ = 24.49, p=0.009), but not 26 weeks ($F_{4,20}$ = 2.37, p=0.084), but further investigation indicated that this was not consistent across all seed meal treatments (Table 4).

Table 4. Analysis of variance (ANOVA) comparing average liverwort cover, excluding plots beneath the tunnel frame, 01.02.10, 19 WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.	
Block	3	1013.0	337.7	2.16	0.124	
Treatment	4	4578.1	1144.5	7.33	<.001	***
Method	1	0.0	0.0	0.00	0.993	
Treatment.Method	4	2803.0	700.7	4.49	0.009	**
Residual	2	3124.8	156.2			
Total	32	9659.6				

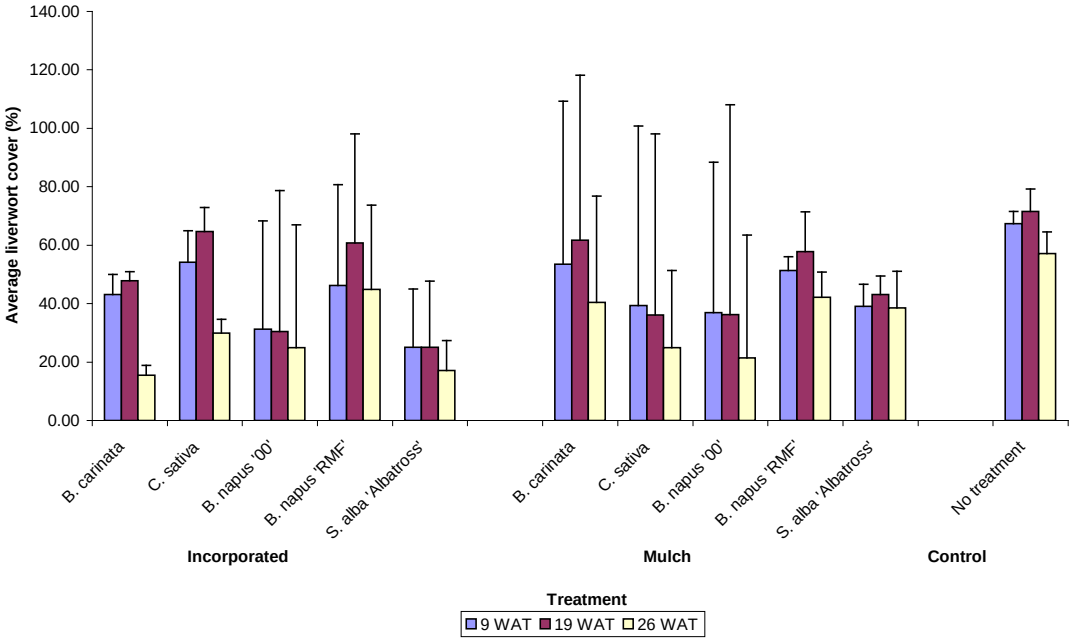


Figure 11. Seed meal suppressive effect excluding plots beneath the tunnel bar (WAT = weeks after treatment)

The seed meals used in this trial had individual glucosinolate profiles (Appendix 1); although the two *Brassica napus* seed meals had similar glucosinolate profiles the proportions of each differed and this could be responsible for the different effects on liverwort establishment. Overall *Synapis alba* and *Brassica carinata* had the greatest glucosinolate contents, but this did not directly translate into the greatest liverwort control in both instances, suggesting a greater influence of individual glucosinolate characteristics on liverwort establishment compared to glucosinolate quantity.

Objective 2: Growing media suppressive effect

Peat treatments had a high level of liverwort infestation from early in the trial as expected. Average pot cover in excess of 82% was recorded in after 11 weeks, 94% after 23 weeks and 99% after 30 weeks (Appendix 4). Liverwort growth was strong and healthy in this trial and the liverwort inoculant had started to spread into immediately adjacent pots across all treatments after four weeks.

Green compost treatments did appear to have an effect, with liverwort slow to establish early in the trial, but after 30 weeks liverwort infestation was comparable to that seen in the peat treatments, with 100% pot coverage in some plots (Appendix 4). Green compost treatments were infested with small black snails (*Oxyloma pfeifferi*) when inspected after four weeks, and the growing media slumped by approximately 10 mm in the pots.

The high level of liverwort that developed in the Growbark® treatment indicated that the surface of the growing media remained moist throughout the trial due to the high level of irrigation applied; it is normally expected that this product would increase drainage, producing a dryer surface and therefore less liverwort cover.

Perlite also increases drainage and one would expect this to reduce liverwort infestation. However, under the moist conditions provided for this trial although liverwort was slower to establish in the perlite treatment than the peat and Growbark®, after 30 weeks liverwort infestations (90% pot coverage) were approaching those recorded in the peat and green compost treatments.

Liverwort was slow to establish in the Sylvafibre® treatments although 78% pot cover was recorded after 30 weeks. The beneficial effect of Sylvafibre® had previously been attributed solely to the improved drainage that it imparts on growing media. The plot in block four did experience a drying effect as it bordered the path, and less liverwort was recorded in this treatment (60%) compared to the other Sylvafibre® plots (77%, 80% and 94%); similarly less liverwort was noted in the perlite (78%) and sterilised loam (63%) plots bordering the path. This was not reflected in the green compost treatment which bordered the path and where 100% liverwort coverage was recorded.

Throughout the trial the sterilised loam showed least liverwort establishment compared to other treatments. Whilst this may show promise in reducing liverwort infestation, the weight and cost of loam may restrict the proportion that could be included in commercial growing media.

Statistical analysis (Table 5) using analysis of variance showed a very highly significant difference in liverwort cover between treatments ($F_{5,15}$ = 11.54, P<0.05) after 30 weeks. Closer inspection of the data indicated that liverwort cover in both the sterilised loam and Sylvafibre® treatments was significantly less than in the green waste, perlite, Growbark® and peat treatments.

Table 5. Analysis of variance (ANOVA) comparing average liverwort cover, 26.02.10, 30 WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.	
Block	3	328.99	109.66	2.36	0.113	
Treatment	5	2686.71	537.34	11.54	<.001	***
Residual	15	698.45	46.56			
Total	23	3714.15				

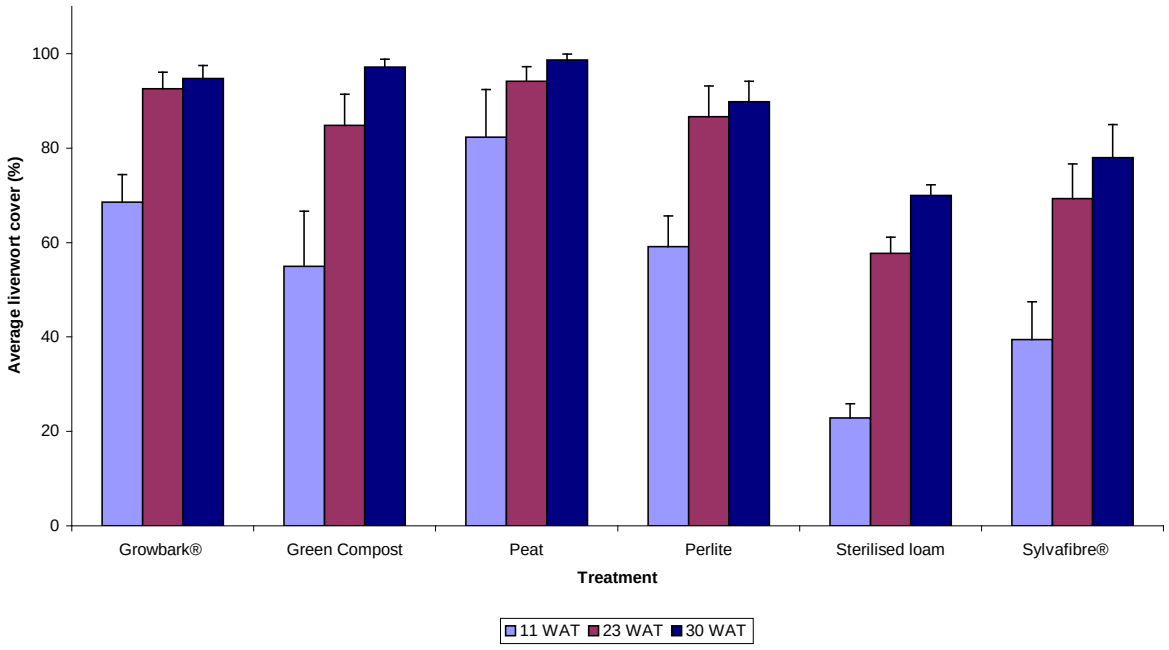


Figure 12. Growing media suppressive effect (WAT = weeks after treatment)

It was noticed during the trial that plots along one edge of the trial area, next to a path (refer to Appendix 2), were drier than the rest as the automatic irrigation system did not provide adequate water to these pots. As it was intended to eliminate the effect of dry growing media surface on liverwort growth the results were re-examined without the data for these plots. The statistical analysis was repeated (Table 6) and again showed a very highly significant difference in liverwort cover between treatments ($F_{5, 9}$ = 32.21, P<0.05). The data indicated that liverwort growth in both the sterilised loam and Sylvafibre® treatments remained significantly less than in the green waste, perlite, Growbark® and peat treatments.

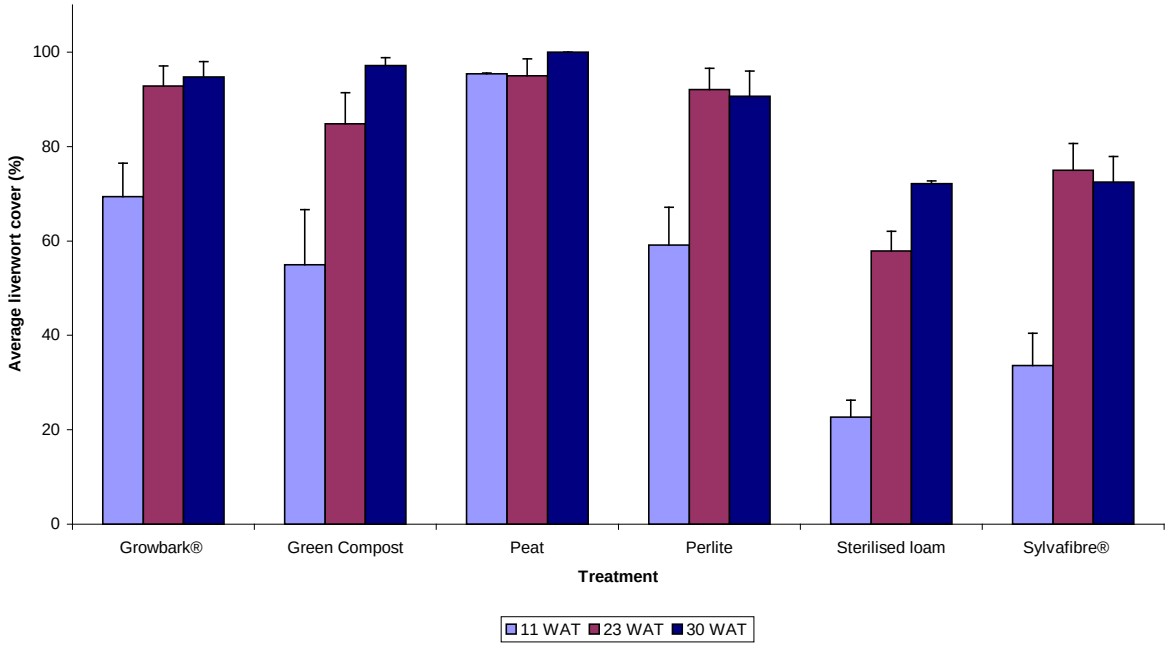


Figure 13. Growing media suppressive effect excluding dry pots (WAT = weeks after treatment)

Table 6. Analysis of variance (ANOVA) comparing average liverwort cover, 26.02.10, WAT

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F. pr.	
Block	3		689.58	229.86	12.52	0.001	***
Treatment	5		2956.58	591.32	32.21	<.001	***
Residual	9	(6)	165.20	18.36			
Total	17	(6)	2836.72				

(m.v. = missing values)

Conclusions

There was a marked difference between the growth of the liverwort at the two sites; in the growing media suppressive effect trial it was lush and green whereas in the seed meal suppressive effect trial it was generally less vigorous with harder foliage. It was likely that this was attributable to the wetter growing conditions maintained for the growing media trial.

The dry conditions of the seed meal trial meant the final results after 26 weeks were not reliable. However, the fungal growth that was isolated could prove interesting for future research. The *Synapis alba*, *Brassica napus* '00' and *Camelina sativa* treatments showed promise. Future research could investigate optimum application rates for liverwort control and combining seed meals to observe any increased effect, however, the effect of seed meals on crop plants also requires investigation.

For short term crops the use of Sylvafibre® may reduce the amount of herbicides applied or the level of pre-sale pot cleaning required. The Sylvafibre® treatment produced promising results, maintaining liverwort cover less than 40% for 11 weeks. The most promising results were obtained using sterilised loam where liverwort cover was less than 23% after 11 weeks. Future work could investigate combinations of treatments compared with irrigation level to establish any significant interactions which could lead to improved liverwort control without resort to chemical products.

Technology transfer

- Article for HDC News, June 2010
- Presentation at IPPS conference planned for October 2010.

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- Seed meal samples were provided by Statfold Seed Oils Ltd (*Camelina sativa* seed meal), Plant Solutions Ltd (Caliente Brand Mustard), ADM Ingredients Ltd ('00' oil seed rape) and Romney Marsh Farms (mixed Oil Seed Rape).
- Growing media amendments were provided by Melcourt Industries Ltd (Growbark® and Sylvafibre®) and Vital Earth Ltd (green compost).

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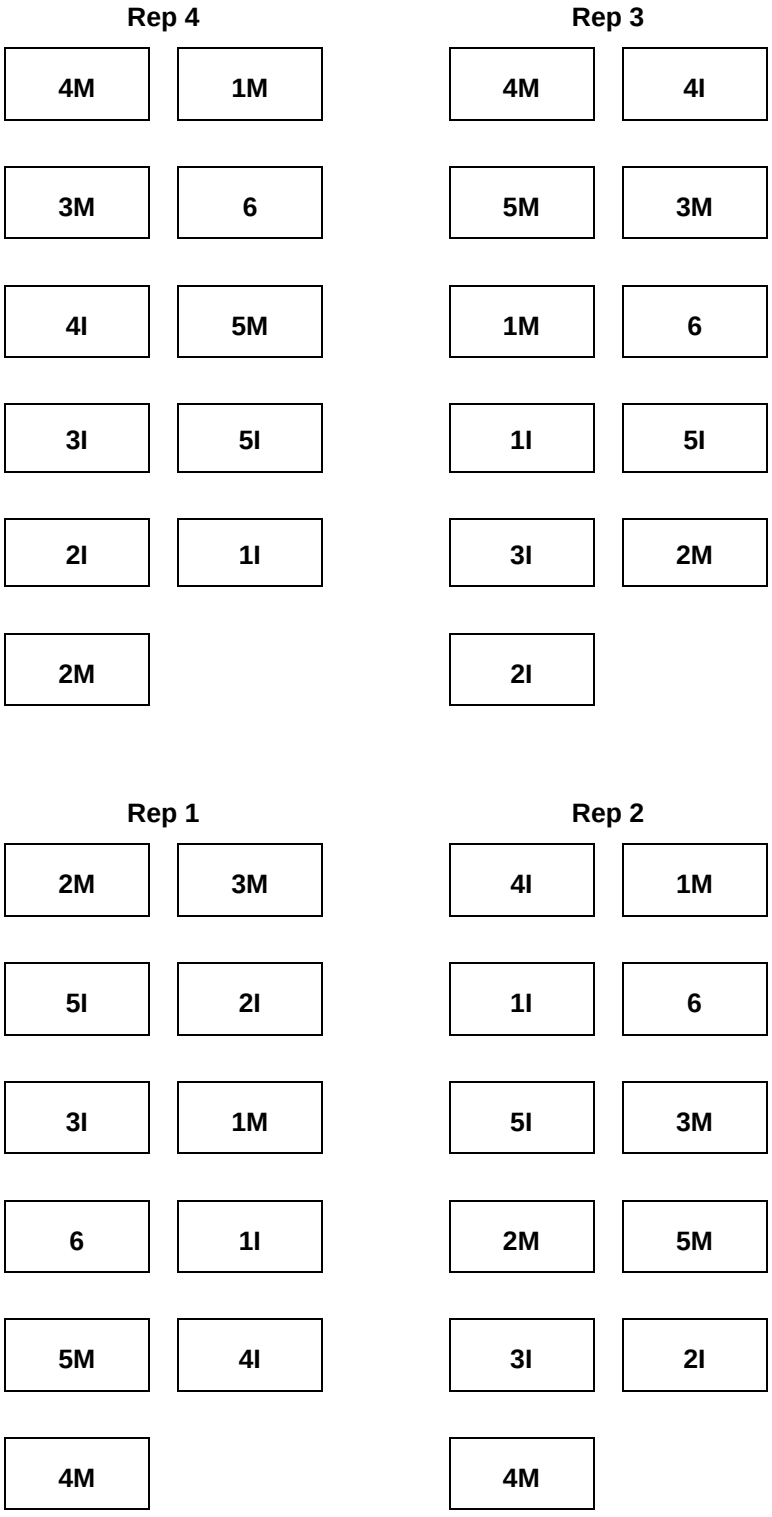
Appendices

Appendix 1. Seed meal glucosinolate analysis

Glucosinolate	Glucosinolate (µmol/g)				
	Brassica carinata	***Sinapis alba* 'Albatross'**	***Brassica napus* '00'**	***Brassica napus* RMF**	***Camellina sativa***
Sinigrin	95.4	0	-	-	-
Glucosinalbin	33.9	187.8	-	-	-
4OH glucobrassicin	2.8	0	0.19	2.12	-
Glucoberin	-	-	0.15	0.89	-
Progoitrin	-	-	6.26	6.32	-
Epi Progoitrin	-	-	0.17	0	-
Glucoraphanin	-	-	0.53	0.54	-
Glucoalyssin	-	-	0.7	0.37	-
Gluconapin	-	-	2.49	2.64	-
Glucobrassinapin	-	-	0.76	1.13	-
Gluconaturtiin	-	-	0.15	0.26	-
Glucocamelinin	-	-	-	-	19.75
9-methylsulfinylnonyl-GLS	-	-	-	-	5.57
11-methylsulfinylundecyl-GLS	-	-	-	-	4.62
Total content	132.2	187.8	11.4	14.3	29.9

Appendix 2. Trial plans

<u>Seed meal suppressive effect</u>

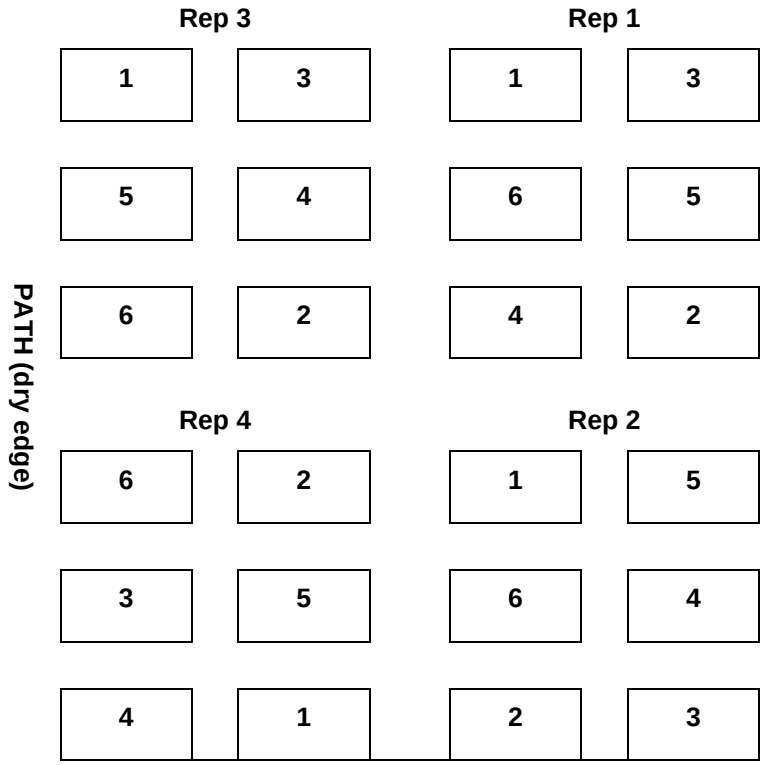


Treatments

1 *Brassica napus* '00' 5 *Brassica carinata*

2	*Brassica napus* RMF	6	No amendment
3	*Camelina sativa*	M	Mulch
4	*Sinapsis alba*	I	Incorporated

Growing media suppressive effect



Treatments

1	Sylvafibre®	4	Sterilised loam
2	Green waste	5	Growbark®
3	Perlite	6	Standard peat

Appendix 3. Seed meal suppressive effect (representative plots)

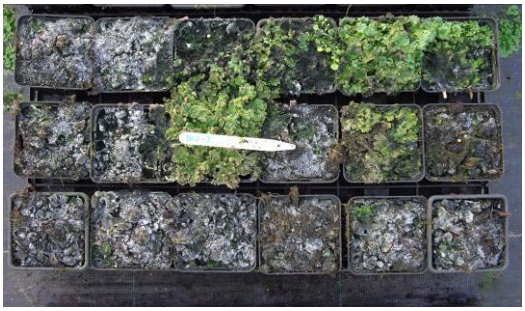
<u>Incorporated treatments</u>

***Brassica napus* '00'**

***Brassica napus* RMF**

Camelina sativa

Sinapsis alba



Brassica carinata

No amendment

Mulch treatments

***Brassica napus* '00'**

***Brassica napus* RMF**

Camelina sativa

Sinapsis alba

Brassica carinata

Appendix 4. Growing media suppressive effect (representative plots)

Growbark®

Green Compost

Loam

Peat

Perlite

Sylvafibre®